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# Comparing topical fluoride application, laser irradiation and their combined effect on remineralisation of enamel<sup>☆</sup>,

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## ABSTRACT

The aim of the study was to verify whether subablative Er:YAG laser irradiation combined with fluoride application could provide a significantly greater inhibition of enamel demineralization histologically using Polarized light microscopy and to compare microhardness of enamel around orthodontic brackets after treatment by either fluoride, laser, or both. Material and methods: eighty freshly extracted premolars were randomly assigned to four groups after bonding: (A) control, (B) irradiated by Er: YAG laser & Voco Bifluoride 10™ varnish, (C) treated by Voco Bifluoride 10™ varnish, (D) irradiated by Er: YAG laser before pH cycle. Caries-like lesions were created by pH-cycling. At the end of the pH cycle, each group was divided into 2 equal subgroups: first subgroup was prepared for microhardness test and examined under polarized light microscope, second subgroup received a second application of therapeutic agents then entered a second pH cycle for another 14 days then teeth were prepared for measuring surface micro hardness and examination under polarized light microscope. Results: group B showed decrease in percentage of lesion depth compared to control group by more than 70% and there was statistically significant difference between it and the other groups. Group D showed increase in percentage of lesion depth with marked loss of enamel surface compared to control group by 29% after 14 days and 9% after 28 days. Moreover group B showed the highest microhardness values and Group D showed the lowest values. Conclusion: In this in vitro study, laser and fluoride combination enhance the resistance of sound enamel around orthodontic brackets more effectively than Er: YAG laser application alone.

## 1. Introduction

Enamel demineralization is particularly common problem during orthodontic treatment, and its treatment is one of the greatest challenges faced by clinicians. The presence of fixed appliances on tooth surfaces with brackets and bands makes it difficult to clean teeth, favour dental biofilm accumulation, in addition to increasing the prevalence of cariogenic bacteria [1,2].

If the process is not reversed at this initial stage, subsurface lesions leading to cavitation will occur. Clinically, the demineralization sites are detected as opaque and porous white spot lesions (WSLs) that may compromise the final result of orthodontic treatment [3,4].

Fluoride is stated to be the most important agent to prevent decalcification and inhibit lesion progression [5]. Recently, more advanced fluoride varnishes with added calcium and phosphate ions have been developed to supplement the amounts of these ions in saliva and

enhance remineralization by fluoride [6].

Laser is among the new techniques to inhibit enamel demineralization and reduce enamel permeability [7]. Recent research has shown that Er:YAG laser can produce positive effects on the increase of enamel acid resistance [8]. Studies have demonstrated that a combined fluoride-laser treatment makes enamel more resistant to acid than does either laser treatment or fluoride treatment alone [9].

The objectives of the present study was to verify whether subablative Er:YAG laser irradiation combined with fluoride application could provide a significantly greater inhibition of enamel demineralization around orthodontic brackets histologically (using Polarized light microscopy) and to compare microhardness of enamel around orthodontic brackets (using Vickers's hardness test) after treatment by either fluoride, laser, or both.

<sup>☆</sup> The study was an experimental laboratory study and was performed on 80 human premolar freshly extracted sound teeth and it was performed at: Faculty of Dentistry, Faculty of Pharmacy, and Faculty of Science - Ain Shams University, National Research institute and Military Specialized Dental Hospital.

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## 2. Material and methods

The study was an experimental laboratory study and was performed at: Faculty of Dentistry, Faculty of Pharmacy, and Faculty of Science Ain Shams University, National Research institute and Military Specialized Dental Hospital.

The study was performed on 80 human premolar freshly extracted sound teeth.

- Inclusion criteria: freshly extracted, sound premolar teeth.
- Exclusion criteria: teeth with caries, hypoplasia, crack defects, and staining or enamel defects. Teeth that had pretreatment after extraction with a chemical agent such as alcohol, formalin, and hydrogen peroxide were excluded.

Before the experimental procedures, the teeth were observed in a stereoscope. Those who had defects in enamel were rejected. Teeth were stored in 0.1% thymol solution in a refrigerator for no longer than one month until the study started [8]. Before bonding procedures, the teeth were cleaned with a scaler to remove calculus and tissue remnants, and the surfaces were polished with a non-fluoridated pumice and washed with deionized water. Buccal surface of each tooth was isolated with an adhesive tape, which was cut similar to the bracket base by a hole puncher to standardize and limit the enamel area exposed to the etching and bonding procedures [10]. Orthodontic brackets were bonded on the buccal surface of the teeth using *biodinamica*® Portugal, biofix according to the manufacturer's instructions.

Teeth were then randomly assigned to the four study groups; twenty teeth each:

- Group A: Control group (No treatment).
- Group B: Irradiated by Er: YAG laser before Voco Bifluoride 10™ varnish then pH cycle.
- Group C: Treated by Voco Bifluoride 10™ varnish before pH cycle.
- Group D: Treated by Er: YAG laser before pH cycle.

After application of the varnish materials or laser exposure or both, all the teeth were allowed to dry for 5 min and the stored in artificial saliva for 12 h then entered the pH cycles [10].

Teeth in laser group in group B&D were mounted on self-cure acrylic blocks, using a plastic circular mold with only their crown exposed before irradiation.

Er: YAG laser was operated at a wavelength of 2.94 μm with a mirror hand piece 2060. The energy output was 80 mJ per pulse, and pulse duration of 200 μs and pulse frequency of 2 Hz was used without water cooling. The laser beam was applied for 10 s in noncontact, focused mode at a perpendicular distance of 4 mm on the buccal surface around orthodontic bracket [8].

Following treatment, all samples were put into pH cycles for 14 days through a daily procedure of de- and re-mineralization. At the end of each 5 consecutive days of cycling, the samples were kept in remineralizing solution for 2 days. The demineralization solution (pH = 4.3) consisted of 2.0 mmol/L of Ca, 2.0 mmol/L of phosphate in buffer solution of acetate 0.075 mol/L. The remineralization solution (pH = 7.0) consisted of 1.5 mmol/L of Ca, 0.9 mmol/L of phosphate, 150 mmol/L of potassium chloride. Each group was cycled in a separate beaker of solution throughout the experiment [11].

Each group was cycled for 6 h in the demineralizing solution, rinsed with deionized water for 10 s, gently dried with absorbent paper and 18 h in the remineralizing solution. Both demineralization and remineralization solutions were changed daily.

At the end of the pH cycle, each group was divided into 2 equal subgroups:

The first subgroup (10 teeth in each group) which received only one treatment then entered one pH cycle. This subgroup was then prepared for microhardness test and examined under polarized light microscope.

The second subgroup (10 teeth in each group) was prepared to receive a second treatment then entered a second pH cycle for another 14 days. At the end of the second pH cycle teeth were prepared for measuring surface micro hardness and examined under polarized light microscope.

### 2.1. Preparation for microhardness test

Subsequent to the pH cycling procedure, teeth were sectioned. The roots were removed 2 mm apically to the cemento-enamel junction and the crowns were hemisectioned vertically into buccal and lingual halves with a 15HC wafering blade on an Isomet low speed saw (Buehler, Lake Bluff, IL, USA) Brackets were removed. Surface microhardness was measured on the middle of the buccal surface cervical to the bracket area using Vickers microhardness tester (Wilson Hardness Vicker tester, Buehler, USA). In the Vickers test, the 100 g load was applied smoothly, without impact, forcing the indenter into the test specimen. The indenter was held in place for 10 s. The physical quality of the indenter and the accuracy of the applied load must be controlled in order to get the correct results [9].

After the load was removed, the indentation was focused with the magnifying eye piece and the two impression diagonals were measured, usually to the nearest 0.1 μm with a filer micrometer, and averaged. Three indentations were made in each specimen 100 μm away of each other.

### 2.2. Preparation for polarized light microscope

The polarized light microscopy is a sensitive technique for assessing de- and remineralization in vivo and in vitro studies. All buccal sections were longitudinally cut into mesial and distal halves using a slow speed diamond saw with copious water spray to create a thin section (approximately 400 μm thick). All the thin sections were then ground with wet 150 grit silicon carbide paper to create sections with a thickness of 100–150 μm. [12] Sections were washed with deionized water and oriented longitudinally on glass cover slides to analyze any surface lesions by a polarized light microscope (PRIOR scientific, PriorLux POL™) at 100× magnification. Photomicrographs of the gingival half of the buccal surface were taken by attached digital camera with fixed magnification. Measurements of demineralized zones were performed using Image J analysis software. For each section, the demineralized area was measured (μm) 3 times, and the mean was calculated and recorded as the lesion depth for that specimen [13].

### 2.3. Data management and statistical analysis

All In-vitro data was collected in the study and was subjected to statistical analysis. Statistical presentation and analysis of the present study was conducted, using the mean, standard deviation, student t-test and Analysis of variance [ANOVA] tests by SPSS V17.

## 3. Results

### 3.1. Surface microhardness was measured after 14 days (for subgroup 1) and after 28 days (for subgroup 2)

A. Surface microhardness results after 14 days (for subgroup 1): were showed in Fig. 1.

Surface microhardness values of all groups showed statistically significant differences (ANOVA ?? < 0.001). Surface microhardness values of group B (Er: YAG laser & Voco Bifluoride 10™ varnish) were found to be the highest followed by group C (Bifluoride 10 varnish) and group D (Er: YAG laser), whereas group A (control) was the lowest.

Group-A values were the lowest values. Range (292–330.66); Mean (306.192) and SD ± (12.882). The difference between group (A),

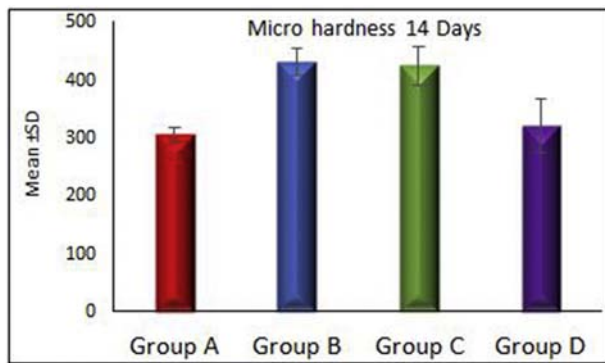


Fig. 1. Bar chart: Multiple comparisons of surface microhardness of subgroup 1 after 14 days.

groups (B and C) was statistically significant ( $P < 0.001$ ). There was no statistically significant differences between groups A and D (Er: YAG laser) ( $P = 0.709$ ).

Group-B values were the highest values. Range (395.066–468.776); Mean (430.770) and SD  $\pm$  23.877. The difference between group B and D was statistically significant ( $P < 0.001$ ). There was no statistically significant differences between groups B and C ( $P = 0.960$ ).

Group-C values were lower than group B values. Range (379.33–468.766), Mean (423.752) and SD  $\pm$  (33.028). The difference between group C, groups A and D was statistically significant ( $P < 0.001$ ).

Group-D values were lower than group B and C values but higher than group A values. Range (268.66–389.233), Mean (321.352) and SD  $\pm$  (46.714).

#### B. SMH results after 28 days (for subgroup 2) were showed in Fig. 2

Multiple comparisons of surface microhardness values of specimens after 28 days were given in Fig. 2. Surface microhardness values of all groups statistically showed statistically significant differences (ANOVA  $P < 0.001$ ). In general, surface microhardness values of group B were found to be the highest followed by group C and group A, whereas group D was the lowest.

Group-A values were higher than group D values. Range (198.4–318.633), Mean (244.063) and SD  $\pm$  (41.222). The difference between group (A), groups (B and C) was statistically significant ( $P < 0.001$ ). There was no statistically significant differences between groups A and D ( $P = 0.913$ ).

Group-B values were the highest values. Range (419.833–456.033), Mean (453.889) and SD  $\pm$  21.862. The difference between group B and D was statistically significant ( $P < 0.001$ ). There was no statistically significant differences between groups B and C ( $P = 0.824$ ).

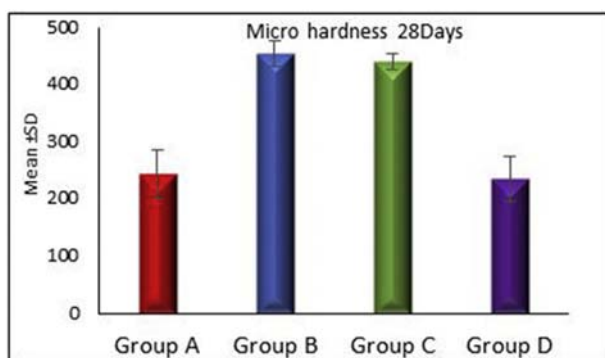


Fig. 2. Bar chart: Multiple comparisons of surface microhardness of subgroup 2 after 28 days.

Table 1

Multiple comparisons of lesion depth measurements of subgroup 1 after 14 days.

Groups	Depth of the lesion (um) 14 Days					ANOVA	
	Range		Mean	±	SD	F	P-value
Group A	92	–	144.33	122.530	±	17.845	43.553  < 0.001 *
Group B	28	–	30	28.667	±	10.155	
Group C	30	–	66.66	43.798	±	14.307	
Group D	101.33	–	197.66	159.197	±	31.101	
TUKEY'S Test							
A&B	A&C	A&D	B&C	B&D	C&D		
< 0.001 *	< 0.001 *	0.007 *	0.799	< 0.001 *	< 0.001 *		

\*Statistically significant differences.

Group-C values were lower than group B values. Range (412.733–468.766); Mean (441.749) and SD  $\pm$  (33.934). The difference between group C, groups A and D was statistically significant ( $P < 0.001$ ).

Group-D values were the lowest than values. Range (197.433–297.66); Mean (234.825) and SD (39.983).

#### 3.2. Lesion depth was measured under Polarized light microscopy and percentage of lesion depth change (increase or decrease) was measured

Descriptive statistics and multiple comparisons of lesion depth of specimens after 14 days were given in Table 1, Fig. 3. Measurements of lesion depth of all groups statistically showed statistically significant differences (ANOVA  $P < 0.001$ ).

Measurements of lesion depth of group B were found to be the least followed by group C then group A (control), whereas group D was the highest.

Group-A measurements: were higher than groups B and C measurements but lesser than group D. Range (92–144.33)  $\mu$ m, Mean (122.530) and SD  $\pm$  (17.845). The difference between group A, groups B and C was statistically significant ( $P < 0.001$ ). The difference between group A and group D was also statistically significant differences ( $P = 0.007$ ).

Group-B measurements: were the least measurements. Range (28–30)  $\mu$ m, Mean (28.667) and SD  $\pm$  (10.155). There was no statistically significant differences between group B and C ( $P = 0.799$ ). The difference between group B, groups A and D was statistically significant ( $P < 0.001$ ).

Group-C measurements: were less than group A and D but more than group B. Range (30–66.66)  $\mu$ m, Mean (43.798) and SD  $\pm$  (14.307). The difference between group B and D was statistically significant ( $P < 0.001$ ).

Group-D measurements: were the highest measurements. Range (101.33–197.66)  $\mu$ m, Mean (159.197), and SD  $\pm$  (31.101).

#### A. Lesion depth measurements after 28 days (for subgroup 2): were showed in Table 2.

Descriptive statistics and multiple comparisons of lesion depth of specimens after 28 days were given in Table 2, Fig. 4. Measurements of lesion depth of all groups statistically showed statistically significant differences (ANOVA  $P < 0.001$ ).

Measurements of lesion depth in group B are found to be the lowest followed by group C then group A, whereas group D was the highest.

Group-A measurements: were higher than groups B and C measurements but lesser than group D. Range (169.33–305.33)  $\mu$ m, Mean (239.597) and SD  $\pm$  (39.429). The difference between group (A), groups (B and C) was statistically significant ( $P < 0.001$ ). There was no statistically significant differences between group A and group D

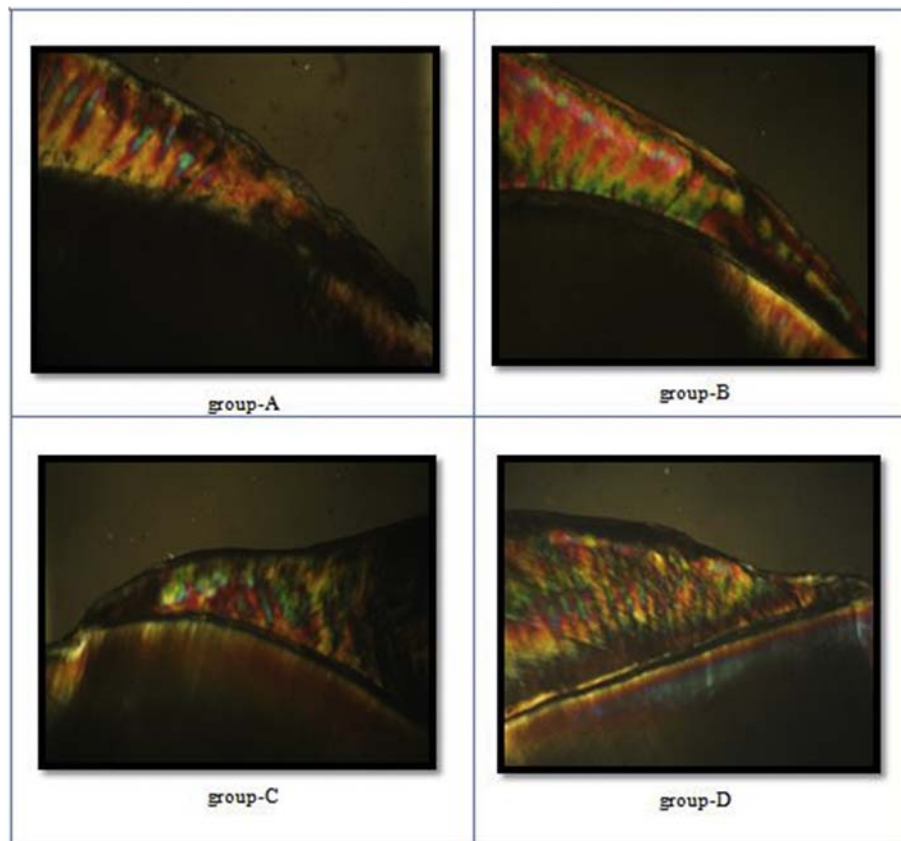


Fig. 3. Polarized light microscopy image of representative lesion after 14 days.

**Table 2**

Multiple comparisons of lesion depth measurements of subgroup 2 after 28 days.

Groups	Depth of the lesion (um) 28 Days					ANOVA	
	Range	Mean	±	SD	F	P-value	
Group A	169.33 – 305.33	239.597	±	39.429	108.834	< 0.001*	
Group B	33.33 – 60	51.464	±	11.675			
Group C	58.66 – 77.33	66.220	±	6.659			
Group D	198.66 – 298.66	261.931	±	27.814			
TUKEY'S Test							
A&B	A&C	A&D	B&C	B&D	C&D		
< 0.001*	< 0.001*	0.314	0.826	< 0.001*	< 0.001*		

\*Statistically significant differences.

( $P = 0.314$ ).

Group-B measurements: were the least measurements. Range (33.33–60)  $\mu\text{m}$ , Mean (51.464) and SD  $\pm$  (11.675). There was no statistically significant differences between group B and C ( $P = 0.826$ ). The difference between group B, groups A and D was statistically significant ( $P < 0.001$ ).

Group-C measurements: were less than group A and D but more than group B. Range (58.66–77.33)  $\mu\text{m}$ , Mean (66.220) and SD  $\pm$  (6.659). The difference between group B and D was statistically significant ( $P < 0.001$ ).

Group-D measurements: were the highest measurements. Range (198.66–298.66)  $\mu\text{m}$ , Mean (261.931), and SD  $\pm$  (27.814).

#### 4. Discussion

Development of White spot lesions during fixed orthodontic

treatment can be prevented by improving patient oral hygiene, enhancing the enamel resistance using topical fluoride and additional new methods as using dental laser [14].

The objectives of the present study were to verify whether sub-ablative Er:YAG laser irradiation combined with fluoride application could provide a significantly greater inhibition of enamel demineralization around orthodontic brackets histologically (using Polarized light microscopy) and to compare microhardness of enamel around orthodontic brackets (using Vickers's hardness test) after treatment by either fluoride, laser, or both.

According to microhardness results and Polarized light microscope analysis: values of group B (Er: YAG laser & Voco Bifluoride 10<sup>TM</sup> varnish) showed highest significant difference in both subgroups (after 14 and 28 days) followed by group C (Bifluoride 10 varnish) compared with control groups and Er: YAG laser groups. However there was no significant difference between laser and control groups in lesion depth measurements (PLM analysis) in subgroup B (after 28 days) but there was significant difference in sub group A (after 14 days).

According to lesion depth percentage, group B (Er: YAG laser & Voco Bifluoride 10<sup>TM</sup> varnish) was able to decrease demineralization depth by 76.6% after 14 days and 78.52% after 28 days followed by group C (Bifluoride 10 varnish). Er: YAG laser showed increase in the demineralization depth by 29.92% after 14 days and 9.32% after 28 days with loss of enamel surface in most of the specimens.

This result was in agreement with other investigation which indicated that there was a significant decrease in demineralization resulted from the combined effect of fluoride and laser compared to fluoride only. There was an average reduction of lesion depth of approximately 40% [15].

The possible explanation for this, as suggested by Dawes and Weatherell [16] could be that laser treatment does not enhance remineralization if not followed by fluoride application, but only inhibits



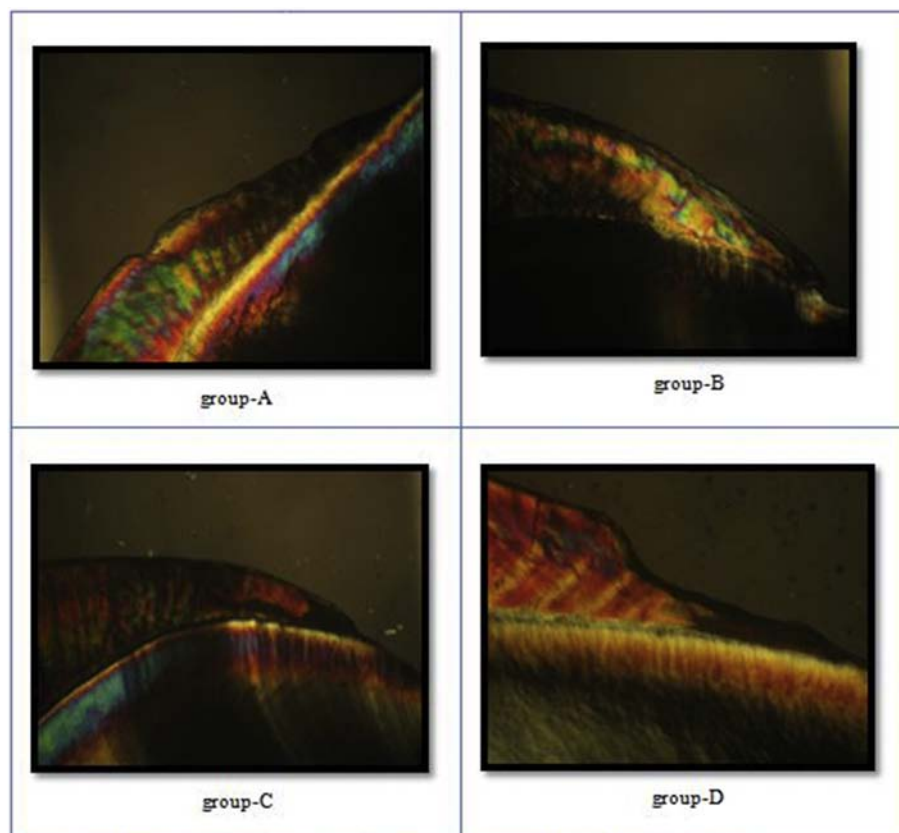


Fig. 4. Polarized light microscopy image of representative lesion after 28 days.

demineralization, and since fluoride interferes physico-chemically with caries development by reducing demineralization and enhancing remineralization, it has shown to have improved caries prevention.

However, there are conflicting results regarding the effect of Er: YAG laser regarding the decrease of enamel solubility [11].

Our data showed loss of hardness on the enamel surface when treated with the sub ablative energy densities of Er: YAG laser only, in agree with our results Apel et al. [17], upon using Er: YAG (2.94  $\mu\text{m}$ ) postulated that subablative Er family lasers can cause fine enamel cracks, a starting point for acid attachment, which may cause deep demineralization and reduce the positive effect of enamel caries prevention. And they concluded that the clinical use of subablative Er laser irradiation to prevent caries formation is not logical.

Also our results were in accordance with Feyza Ulkur et al. [10], who suggested that Er: YAG laser was not effective in preventing demineralization.

On the contrary Correa-Afonso et al. [18], who indicated that Er:YAG laser was efficient in preventing demineralization at a 4 mm distance using water cooling. Also on the contrary Liu J. et al. [19], found that sub ablative low-energy Er: YAG laser irradiation can significantly prevent enamel demineralization potentially through the retardation of enamel diffusion.

We choose two applications of the therapeutic agents in this study in relevant with Manuel Restrepo et al. [20], which proved that two application of NaF varnish were effective in controlling WSLs adjacent to orthodontic brackets. Also Gustavo Vivaldi-Rodrigues et al. [21] showed that periodic fluoride varnish application during orthodontic treatment can help to reduce the incidence of white spot lesions by 44.3%. Also Farhadian N [22] reported reduction in demineralization depth by 40%.

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